Optimisation of plant protein and transglutaminase content in novel beef restructured steaks for older adults by central composite design

Sephora Baugreet, Joseph P. Kerry, André Brodkorb, Carolina Gomez, Mark Auty, Paul Allen, Ruth M. Hamill

PII: S0309-1740(17)31462-6
DOI: doi:10.1016/j.meatsci.2018.03.024
Reference: MESC 7510

To appear in: *Meat Science*

Received date: 20 November 2017
Revised date: 26 March 2018
Accepted date: 26 March 2018

Please cite this article as: Sephora Baugreet, Joseph P. Kerry, André Brodkorb, Carolina Gomez, Mark Auty, Paul Allen, Ruth M. Hamill , Optimisation of plant protein and transglutaminase content in novel beef restructured steaks for older adults by central composite design. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Mesc(2018), doi:10.1016/j.meatsci.2018.03.024

Optimisation of plant protein and transglutaminase content in novel beef restructured steaks for older adults by central composite design

Sephora. Baugreet[1, 2], Joseph P. Kerry[3], André Brodkorb[2], Carolina Gomez[2], Mark Auty[2], Paul Allen[1] and Ruth M. Hamill[1]

[1] Food Quality and Sensory Science Department, Teagasc, Ashtown Food Research Centre, Ashtown, Dublin 15, Ireland

[2]Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland

[3]Food Packaging group, School of Food and Nutritional Sciences, Food Science Building, University College Cork, Ireland

Email: Ruth.Hamill@teagasc.ie

Abstract

With the goal of optimising a protein-enriched restructured beef steak targeted at the nutritional and chemosensory requirements of older adults, technological performance of thirty formulations, containing plant-based ingredients, pea protein isolate (PPI), rice protein (RP) and lentil flour (LF) with transglutaminase (TG) to enhance binding of meat pieces, were analysed. Maximal protein content of 28% in cooked product was achieved with PPI, RP and LF. Binding strength was primarily affected by TG, while textural parameters were improved with LF inclusion. Optimal formulation (F) to obtain a protein-enriched steak with lowest hardness values was achieved with TG (2%), PPI (8%), RP (9.35%) and LF (4%). F, F1S (optimal formulation 1 with added seasoning) and control restructured products (not containing plant proteins or seasonings) were scored by 120 consumers' aged over-65 years. Controls were most preferred ($P<0.05$), while F1S were least liked by the older consumers. Consumer testing suggests further refinement and optimisation of restructured products with plant proteins should be undertaken.

Keywords: Older adults, restructured beef steak, plant proteins, transglutaminase, microstructure, microscopy

1. Introduction

The promotion of healthy ageing is an important challenge globally (Schoufour, Voortman, Franco, & Kiefte-De Jong, 2017) and nutrition is increasingly recognised to play a key role in supporting the older population to live longer and healthier lives. Older adults present specific nutritional needs and elevated intakes of high quality protein which are highly digestible and containing all essential amino acids, as well as specific macro- and micro-nutrients are required. For example, recent studies have shown that the consumption of protein-enriched foods by older adults may increase longevity (Beelen, De Roos, & De Groot, 2016; Stelten et al., 2015). As we advance in age however, sensorial acuity and appetite are both reduced, as well as the ability to chew tougher-textured foodstuffs (Baugreet et al., 2017). Therefore, maintaining a healthy diet and adequate nutritional intake in this age group can be challenging. Consequently, there is a requirement to assess potential solutions; both to provide adequate nutrition in smaller portion sizes, typically consumed by older people, and to optimise the characteristics and appeal of foodstuffs to stimulate interest and promote intakes. With changing demographics reflected in a growing cohort of older (>65, >80 years) adults across Europe and worldwide, the requirement to nutritionally- and sensorially-tailor food products for this group of citizens is increasingly recognised.

Fresh beef is a notable source of high-quality protein, essential amino acids, including the branched chain amino acids, which support muscle protein synthesis, as well as essential micronutrients such as iron, zinc, selenium and B vitamins, namely; niacin, riboflavin, thiamine, B_6 and B_{12}. Several studies have reported the negative health impact around the consumption of red and processed meats and the increased risk of age-oriented conditions (i.e., CVD, cancer) (Bouvard et al., 2015, IARC., 2015). Others have reported a positive relationship between meat consumption and healthy ageing (Kappeler, Eichholzer, & Rohrmann, 2013; Rohrmann et al., 2013). Among older Europeans, a dietary protein intake of 0.83 g/kg/day has been highlighted as being insufficient, and the reduced bioavailability of protein in this cohort (Deer & Volpi, 2015), puts them at risk of developing bone health issues. Furthermore, adults aged 65+ are frequently affected by an age-related condition known as sarcopenia, which leads to muscle wastage due to the decrease in lean body mass (Gariballa & Alessa, 2013). A combined increase in protein

intake and physical activity could help maximise muscle protein synthesis (MPS) and functional status, hence optimising longevity.

Restructuring of meat using lower-value cuts e.g. beef chuck (shoulder) has been an important approach in developing palatable and nutritious value-added meat products (Lepper-Blilie, Berg, Germolus, Buchanan, & Berg, 2014). Plant proteins have been widely utilised in many foods, principally for their nutritional composition and their physiological and technological functionality. Plant proteins such as pea, rice and lentil, among others, are becoming more popular as they are classified as more sustainable protein sources, are typically non-allergenic and non-genetically modified in comparison to soy and can be processed to clean label status. They also have potential to boost protein intakes in products targeted at older people. A process technology known as the PiVac system allows whole muscles or pieces of meat to be tightly wrapped into a chamber of elasticated packaging, which effectively improves tenderness and delivers a uniformly shaped product (Baugreet et al., 2017, Taylor & Hopkins, 2011). PiVac technology, complemented with added plant proteins, offers the opportunity to develop soft textured and enriched meat products for older consumers (Baugreet et al. 2017).

Response surface methodology (RSM) is a statistical tool that generates mathematical models allowing optimised products to be developed based on influencing response factors (Cetiner, Acar, Kahraman, Sanal, & Koksel, 2017). The aims of this study were; 1) to optimise the levels of pea protein isolate (PPI), rice protein (RP), lentil flour (LF) and transglutaminase enzyme (TG) so as to produce an optimised and technologically-acceptable formulation and 2) using an *in vitro* digestion model, in association with microscopy, to better understand the structural changes induced by gastric and intestinal digestion. Finally, 3) the acceptability of restructured beef steaks was assessed in a consumer study of 120 consumers aged greater than 65 years old.

2. Materials & Methods

2.1 Preparation of restructured beef steaks

Coarsely ground beef chucks (95% visual lean) were mixed without water and ingredients for 2min at a speed of 250rpm/min (Stephan Mixer, SohneGmbH & Co, 3250 Hamln, Germany).

Half of the chilled distilled water was incorporated and mixed for 2min. Activa®^{EB} (TG) (Ajinomoto Europe, Hamburg, Germany) was dissolved in the remaining water, added to the mixture and mixed for 3min. Finally, vitamin/mineral premix [selenium, vitamin A, zinc, vitamin B_6, vitamin B_{12}, vitamin C, vitamin E, vitamin K_1, folic acid] (Vitablend, Wolvega, The Netherlands) (20mg/100g of meat) was added and mixed for 1min. Using a hand crank filler, each formulation was stuffed into a plastic casing of 100mm in diameter (Food Processing Technology, Tallaght, Ireland). Once stuffed, it was clipped at both ends and PiVac was applied. The mechanism of PiVac was discussed in detailed in a previous study by Baugreet et al. (2017). Meat logs were initially placed in a chill environment (4°C for 16-18h) to provide adequate bind and subsequently stored in a freezer (-20°C for 24h) before slicing into steaks (1.5cm thick, ~107g). Restructured beef steaks were individually vacuum packed and stored frozen (-20°C) until use.

2.2 Compositional Analysis

Each restructured beef steak was thawed at 4°C overnight, then finely grounded in a Robot Coupe Blender before analysis. Fat, moisture, protein (*N x 6.25*) and ash as defined by AOAC official method 992.15, 1990 and ISO 936:1998 were evaluated as modified by Baugreet et al., (2016).

2.3 Cooking loss, thaw loss and thiobarbituric acid analysis

Restructured beef steaks were cooked overnight (12h) in a circulating water bath (Lauda M40, Delran, New Jersey) set at 75°C**.** Cooking loss was calculated by the difference in the weight of restructured beef steaks before and immediately after cooking as described by Tobin, O'Sullivan, Hamill, and Kerry (2012). Thawing loss was calculated as weight loss (%) taking into account the initial weight of frozen steaks. Thiobarbituric acid-reactive substances (TBARs) was carried out as described by Siu & Draper, (1978), as modified by Baugreet et al., (2016).

2.4 Texture profile analysis (TPA) and bind strength

Texture profile analysis (TPA) was carried out on cooked restructured beef steaks based on a method described by Bourne (1978) and Baugreet et al. (2016). Samples were cooked as described in section *2.3*. Six cores of 18 mm cylindrical samples were taken at random in three cooked restructured beef steaks.

The binding strength of six cooked restructured beef steaks was determined using a Texture Analyser mounted with a spherical probe (Stable Micro Systems, Surrey, UK) as described by (Baugreet et al., 2017). One core of 3.5 cm cylindrical samples was taken from each cooked restructured beef steaks.

2.5 Colour measurements

Colour parameters of raw and cooked restructured beef steaks were measured using the CIE *L*a*b** system with a dual beam xenon flash spectrophotometer (UltraScan XE, Hunterlab., Inc., Reston, VA) in reflectance mode as described previously by Baugreet and colleagues (2016). Each package was opened and left to bloom for 30 min before measurements were taken. All values were the mean of six independent measurements obtained at random from triplicate restructured beef steaks.

2.6 Experimental design and statistical analysis

Response surface methodology (RSM) analysis was performed to examine the inclusion of four compositional variables: TG (X_1), PPI (X_2), RP(X_3) and LF (X_4) on product performance, using Design Expert 10 (Stat-Ease., USA). The experimental design, based on a central composite design (CCD), consisted of 16 factorial runs, 8 axial runs and 6 repetitions at the centre point, resulting in 30 runs, and corresponding levels are shown in Table 1. The experimental sequence was randomised to minimise the effects of uncontrolled factors. The dependent variables (responses) were compositional analysis (moisture, fat, protein, ash), bind strength, cooking loss, lipid oxidation at day 0 and 30, instrumental colour (*L**, *a**, *b**) analysed on both raw and cooked restructured steaks. Textural parameters (hardness, cohesiveness, chewiness, gumminess, springiness) were analysed on cooked steaks and thaw loss on raw restructured beef steaks.

The mathematical models were evaluated for each response using multiple linear regression analysis. Modelling started with the development of a quadratic model including linear, squared and interaction terms. The significant terms of each response of the model were reported by analysis of variance (ANOVA). A polynomial quadratic regression equation (Eq. 1) was used to determine the effects of the four factors. Where Y is the dependent variable (moisture, protein, cooking loss, etc.), β_0 is the constant, β_i; β_{ii} and β_{iii} are regression coefficients and X_i, X_j are levels of the independent variables. The model adequacies were checked by R^2 and adjusted R^2 (Meyers & Montgomery, 2002).

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j$$

Eq. 1: Polynomial quadratic regression equation

All the determinations of the response variables were carried out in triplicate. Significance was judged by determining the probability level of the F-Statistic calculated from the data was less than 5%. Response surface plots were drawn out to show the simultaneous effects of TG, PPI, RP and LF on the experimental dependent parameters. The adequate precision, which measures the signal-to-noise, was more than four for all responses, which is highly desirable.

2.7 Optimisation and validation

The numerical optimisation technique within the Design-Expert software was used for simultaneous optimisation of the responses. The desired goals for each variable (TG, PPI, RP, LF) and response (protein, moisture, L^*, a^*, cook loss, hardness, cohesiveness, chewiness, springiness, bind strength) were chosen. To validate the RSM model, restructured steaks based on the optimised levels of ingredients were prepared**,** and the experimental values for each response were compared to the predicted data from the RSM models. Results obtained were statistically compared to the values predicted by the mathematical model using the accuracy and bias factors.

2.8 In vitro digestion

Cooked optimised formulation restructured beef steaks (as per section *2.3* and *2.7*) were subjected to the *in vitro* digestion performed according to a standardised method, specifically designed for studying simulated gastrointestinal digestion (Minekus et al., 2014). Digestions consisted of an enzymatic digestion simulating the mouth, stomach and small intestine. Briefly, meat samples of approximately 2.5g were subjected for using human the oral digestion 'chew and spit' method, and re-weighed again. Each sample was then placed in a centrifuge tube and placed on ice until use. About 2.4ml simulated gastric fluid (pH 3) at 37°C was added to the tubes, followed by porcine pepsin activity (to achieve 2,000U/ml) and 1.5µl of calcium chloride (150µM final concentration). Each sample was followed by an acidification step to pH 3 using 2M HCI, thus initiating the gastric digestion process. At the end of gastric digestion, to inactivate pepsin and to simulate digestion in the small intestine pH was adjusted to 7. The digestion samples were preserved after 0, 30, 60, 90, 120 min of incubation at 37°C in a rotator. To end the gastric phase, 1M sodium hydroxide was used to increase pH to 7 and snap-frozen in liquid nitrogen. For the intestinal phase, 3.8ml of simulated intestinal fluid was added; 0.6ml of bile salt (10µM final concentration) was added, as well as 1ml of pancreatin to achieve a trpsin activity of 100U/ml. The pH was further increased to pH 7 for each sample using 1M sodium hydroxide and placed in a 37°C incubator on a rotator. The final volume of each digested sample (digesta) was approximately 12ml. Upon completion of the intestinal phase, an inhibitor (Pefabloc®) was added at 20µl.

2.9 Microstructural changes observed during in vitro digestion

Optimised formulations were subjected to *in vitro* digestion as reported above, except that, during the gastric and intestinal phase, 1ml aliquots of digesta were removed after 120min for microstructural analysis. For gastric and intestinal meat samples, 20µl of sample was placed on a microscope slide and 5µl of Nile Blue (aqueous, 0.1% w/w) added to the sample. A coverslip was placed on top of all samples and they were imaged in a Leica SP5 confocal scanning laser microscope (Leica Microsystems GmBH, Mannheim, Germany). Dual channel images were

acquired with a x10, x20 and x63 objectives, using a 488nm argon ion laser to image fat (green) and a 633nm helium neon laser to image protein, myofibrils and connective tissue (red).

2.10 Consumer sensory evaluation

Cooked optimised formulations (as per section *2.3* and *2.7*) were evaluated by a panel comprised of 116 older adults (>65 to 80+ years old, 62 females, 54 males) recruited by the Agri-Food and Biosciences Institute, Belfast, Northern Ireland, taking into account demographic information (age group, former occupation) and product related questions (labels, openability of packaging). Additional treatments (Control (C) and F1S) were prepared for comparison as described above. For F1S a seasoning blend was added at 2g/100g. The seasoning blend contained tomato powder (30%), basil (20%), ground coriander (15%), ground sage (15%) and rosemary (20%) purchased from Redbrook, Dublin, Ireland. Evaluations were performed in individual booths prepared as described by ISO 8589 (2008). Unsalted crackers and water at room temperature were provided to clean palate between samples. The sensory analysis was carried out using a 10cm hedonic scale on which the assessors evaluated various liking attributes: aroma, tenderness, juiciness, flavour and overall liking (Table 2).

For the consumer study, an ordered logistic regression (proportional odds model) with random effects was carried out to analyse the data. It was fitted using maximum likelihood estimation using the Meologit command in Stata (v14.2).

3. Results and Discussion

3.1 Composition and processing characteristics

Compositional data for ash (raw and cooked) and protein (cooked) could not be fitted**,** and data are not presented here.

The consumption of protein-enriched foods is beneficial in preventing age-related muscle loss (sarcopenia), improving bone health and enhancing the quality of life among older adults (Baugreet, Hamill, Kerry, & McCarthy, 2017; Baugreet et al., 2016). Protein content in raw

restructured beef steaks was driven ($P<0.01$) by the addition of RP (Table 3), achieving a maximum of 28% protein in each 100g beef steak (Fig. 1a). An intake of at least 25-30g of high-quality protein per meal has been suggested in the recent literature, to maximise muscle protein synthesis and maintain muscle mass in older adults (Beelen et al., 2016). Therefore, the maximal protein content in a portion controlled product (28g/100g restructured beef steaks) observed here would be beneficial in assisting older adults to reach targeted protein requirements. The enriched protein content in the raw samples (25%) was maintained throughout the cooking process, and indeed cooked samples from all formulations had numerically higher protein content compared to raw (30%) however**,** this difference was not significant. It is clear in any case that the enriching plant proteins were retained in the meat matrix after cooking.

Moisture content for raw and cooked restructured beef steaks ranged between 61.6%-70.8% and 59.9%-68.8% respectively, and both negatively correlated with RP inclusion (Table 3). RP remained a significant model term after cooking. The surface plots showed that inclusion of RP at >7.5% decreased moisture levels (Fig. 1b/1c). A 3.01% reduction (67.9% to 64.9%) in moisture levels was observed (RP at 7.5%) after cooking. The effect of cooking and temperature on rice protein has been shown to cause heat induced coagulation resulting in reduced moisture (Nehete, Bhambar, Narkhede, & Gawali, 2013).

The fat content of cooked restructured steaks was mainly affected by the interaction between PPI and RP (Table 3). Fat reduction is caused by PPI (<7% PPI) being more pronounced as RP increased (Fig. 1d). Usually**,** proteins as isolates (>80% protein) or concentrates (30-80%) are employed in low-fat meat processing (Petracci, Bianchi, Mudalal, & Cavani, 2013). Hence, these ingredients are useful in the development of healthier low-fat processed meat products.

3.2 Textural profile analysis (TPA) and bind strength

The inclusion of all four ingredients; PPI, RP, LF and TG in restructured beef steaks significantly affected their textural characteristics ($P<0.05$) (Table 4). When LF inclusions were increased (1.5-4%), this caused a decrease in hardness (900.96) and gumminess (183.65) (Fig. 2a-b). The likely reason for this is the lower protein content in LF, which probably resulted in weaker gel formation, and decreased the product's resistance to compression. There was an

interaction between PPI and *LF which resulted in a reduction in hardness values (816.10) caused by LF (at 4%) this being more pronounced with the addition of PPI (at 7%) (Fig. 2a). Adebiyi and Aluko (2011), found that pea protein isolate formed a paste instead of a rigid gel structure in products. Hence, the interaction of PPI and LF produced an enhanced softening effect in the restructured beef steaks. Springiness and chewiness increased in a linear fashion with increasing TG (>1-<2.5%) (Fig. 2c/2d). A similar effect was observed by Colmenero, Ayo, and Carballo (2005), where TG in combination with caesinate led to springier and chewier frankfurter sausages. Cohesiveness signifies the degree of difficulty in breaking down the internal structure of meat. RP in conjunction with LF at minimal and maximal inclusion levels (RP: 10%; LF: 1.5%) and (LF: 4%; RP: 7.5%) decreased cohesiveness values in restructured steaks (P<0.001) (Fig. 2e).

Binding strength appeared to increase gradually with the addition of 1-2.5% TG from (6056-7607g), however this change was not significant. Previously, TG has been used mostly with the addition of salt (1-3%) to achieve efficient binding (Sun, 2009). Here, perhaps the effect produced by the added protein ingredients is masking the binding associated with TG. It is noteworthy that our results suggest that the use of TG may not be essential to achieve acceptable techno-functional properties of a restructured product containing protein-rich ingredients. This could have positive implications for achieving clean label status for restructured meat products.

3.3 Cooking loss, thawing loss and lipid oxidation

Cooking loss is an important parameter in meat products as it measures water and fat binding after protein denaturation and aggregation during the cooking process (Hayes, Stepanyan, Allen, O'Grady, & Kerry, 2011). A reduced cooking loss in restructured beef steaks can be achieved at a PPI and LF content of at least 10% and 4%, respectively, indicating that these ingredients are useful in retaining moisture in the product during cooking (Fig. 3a/3b). These results are consistent with the moisture data, wherein no significant decline in moisture was observed, with increasing PPI and LF. These plant proteins may form a well-structured protein matrix or a gel which then traps water and prevents its release (Ustunol, 2014).

Fig. 3c and 3d illustrate TBARs values of raw restructured steaks at day 0 and 30. The model term RP and quadratic term LF^2 were significant ($P<0.05$) for lipid oxidation at day 30 (Table 4). Maximum and minimum values for lipid oxidation were found at approximately 7.5% (0.64) LF and 10% (0.51) LF, respectively. The interaction of PPI and RP was the factor that most affected lipid oxidation at day 0. Maximum TBARs values were observed from PPI*RP (PPI 5.5%; RP 7.5% or PPI 8%; RP 10%), 0.66 and 0.67, respectively. However, PPI at 6.75% and RP 8.75% produced minimum TBARs values (0.41). This is an indication that meat alone was affected by lipid oxidation during processing which is in line with a previous study by Baugreet et al. (In Press). A significant quadratic term for LF is seen as a curved line (Fig. 3c). These results are in line with Akcan, Estévez, and Serdaroğlu (2017), where the use of whey protein films in meatballs resulted in lower TBARs values during 30 days frozen storage. Our result indicates that lipid oxidation was effectively retarded by combining PPI, RP and LF during frozen storage time and did not exceed the suggested sensorial threshold of 1 mg MDA/kg meat (Alakali, Irtwange, & Mzer, 2010).

There was no significant effect on thaw loss for restructured beef steaks and data are not presented here.

3.4 Colour parameters

The parameters L^* and a^* (raw), and L^* (cooked) (Table 5) were significantly affected by the formulations ($P<0.01$). A maximal increase of 51.85 in L^* values were observed in raw restructured steaks with an inclusion level of LF of 4% (Fig. 4a). Interactions for a^* observed between TG*LF, PPI*RP, PPI*LF and RP*LF were significant. The interaction between PPI and *RP (when PPI 8%; RP 7.5% or PPI 5.5%; RP 10%) and RP*LF (when RP 10%; LF 1.5% or RP 7.5%; LF 4%) will produce redness value of 9.6 (Fig. 4b and 4c). However, TG*LF (TG 1%; LF 1.5% or TG 2.5%; LF 4%) and PPI*LF (PPI 5.5%; LF 1.5%) produced maximal redness values (9.7) (Fig. 4d/4e). Our results indicate that non-meat ingredients influenced colour parameters. For cooked restructured steaks, RP and LF showed a significant effect on L^*. When RP and LF increased from 7.5-10% (52.49-54.37) and 1.5-4% (52.78-54.23) respectively L^* values increased (Fig. 4f/4g). The other colour parameters a^* and b^* were not significantly affected and

data are not presented. Consumer choice for acceptable beef quality is often based on a bright red colour (Holman, van de Ven, Mao, Coombs, & Hopkins, 2017). Hence, it can be concluded that TG*LF and PPI*LF may enhance the visual appearance of raw restructured beef products at point of sale.

3.5 Optimisation and validation of restructured beef steaks formulation

Optimisation was applied within the experimental variables (TG, PPI, RP and LF) to identify optimum formulations for the restructured steaks for desired experimental goals. Using the numerical optimisation technique in the software (Design Expert 10, Stat-Ease Inc., Minnepolis, MN, USA), all chosen responses were either maximised, minimised or set within limits (Table 6). This tool predicted two similar optimised formulations (F1 & F2) which maximised protein and generated predicted values for each dependent variable (Table 6) which had acceptable overall desirability levels (0.50) for pea protein isolate, rice protein and lentil flour addition. These consisted of F1) PPI 8%, RP 9.35% and LF 4% and F2) PPI 8%, RP 9.39% and LF 4%. Validation is a major step in RSM to assess that the model and limits are accurate and precise. Table 6 shows the performance of the model indices when optimal formulations were composed and analysed in relation to their technological performance. The models showed a good fit for both optimised formulations as demonstrated by the accuracy and bias factors which are close to 1.00 for the key responses. Our results showed that a central composite design approach can be applied to develop a complex optimised enhanced protein, softer restructured beef steak formulation with acceptable technological properties that could have great potential for application to enhance the availability and intake of high quality protein in the diets of older adults.

3.6 Product microstructure and digestive behaviour visualised using microscopy

Representative confocal images of beef steaks before and after *in vitro* digestion are presented in Fig. 5. Restructuring meat with plant proteins and TG can dramatically alter the structure of a

meat system and as expected in the images of the control, F1 and F2 at each step (cooked, gastric and intestinal phase) were quite different.

The image of the cooked control illustrated an intact muscle fibre structure, with perimysium evident between the fibre bundles, demonstrating that the cooking process did not disrupt the fibre structure (Fig. 5A). In F1 and F2 the cooked restructured beef samples showed large protein aggregates in addition to the muscle pieces as well as dispersed fat globules, which probably represent the heat-treated pea protein isolate, rice protein and lentil flour ingredients added to the formulation (Fig. 5D, G).

3.6.1 Gastric and intestinal phase

Following the addition of pepsin in the gastric phase, all products demonstrated a disruption to the fibre structure, possibly due to extensive breakdown of the collagenous perimysium and endomysium. The addition of plant-based ingredients in F1 and F2 resulted in distinct non-meat protein islands shown to be dispersed within the meat protein matrix (Fig. 5E, H). Irregular shape fat globules were dispersed in all treatments (Fig. 5B, E, H). However, in Fig. 5H, fat appeared more abundant. Changes after the intestinal phase showed the fibre bundle structure in the control sample had almost completely broken down, with myofibrillar striations almost absent (Fig. 5C). In F4 and F6 (Fig. 5F, I), a complete disintegration of the myofibrils can be seen. However, the presence of some granular and chain-like aggregate was evident on the micrographs which could be associated either with the integral meat proteins or with the ingredients added (Fig. 5F, I). The characteristics of plant-based ingredients (cell wall components, arrangement of starch granules) may impair the access of gastrointestinal enzymes during digestion (Singh, Kaur, & Singh, 2013). The combination of meat protein and ingredients used in this study certainly appears to have altered the overall digestive behaviour of the experimental formulations.

3.7 Consumer evaluation

Although instrumental analysis provides important information on the physico-chemical properties of the product, the intricacy of the changes due to the inclusion of plant-based ingredients to restructured beef steaks in relation to acceptance could only be perceived by discrimination and consumer tasting (Aleson-Carbonell, Fernández-López, Pérez-Alvarez, & Kuri, 2005; Orla, Barbara, Peter, & David, 2004). Sensory evaluation of the optimised restructured beef steaks by the taste panel of over-65s revealed significantly different ($P<0.05$) scores for aroma liking, juiciness, flavour liking and overall liking (Fig. 6a) among treatments. Although panellist rated tenderness slightly higher for F1, this score was not significantly different ($P>0.05$) from C and F1S. Overall liking for all restructured products tested was quite low as all samples scored below 30%, but overall liking for control samples scored higher ($P<0.05$) than products with plant proteins. A breakdown by age subgroups (65-69, 70-74, 75-79 and 80+ years) to examine the influence of different descriptors were also analysed. When the overall means scores for sensory attributes was examined within each age subgroups, among the 80+ age category aroma, flavour and overall liking were not significantly different for any product (Fig 6b). Only the oldest cohort (75 to 80+) in this study rated flavour liking favourably for all samples (Fig 6c). When compared to the 65-69 age cohort, this result shows that age-associated changes in chemosensory perception is more pronounced as one advances in age leading to difficulty in discriminating between samples with respect to flavour manipulation. There are likely to be complex effects on flavour and aroma when diverse plant-based ingredients are added to the restructured product and this may have influenced aroma and flavour scores, which were lower for the experimental formulations. The seasoning blend used in this study comprised of 70% bitter-floral characteristics, with distinctive citrus notes, while only 30% was derived from umami flavour profile. This could explain why the specific seasoning flavour combination was not particularly liked. Elsewhere, when gari was added to beef burgers, products were less liked (Akwetey & Knipe, 2012). Therefore, the next step might be to enhance the flavour with an enriched savoury blend.

The hypothesis for adding seasoning to the product, was that chemosensory perception and acuity would be reduced in the cohort under study hence they may perceive standard products as bland. Interestingly, the formulation with added plant proteins and seasonings were more appreciated among the 75 to 80+ age cohort. This finding agrees with the results reported by

Griep, Mets, & Massart (2000) and Mathey, Siebelink, de Graaf, & Van Staveren (2001). Older adults with impaired odour perception might benefit from flavour enhancement in food since the increased flavour either by plant proteins and seasonings may compensate for their sensory loss (Griep, Mets & Massart, 1996). In line with this study, a tendency was observed that participants above 75 years old without preference for either three samples may have a poorer odour perception in comparison to the other age groups studied showing a clear preference among samples.

This work offers direction and guidance to future optimisation which could focus on enhancing juiciness of the product, and modifying the seasoning where possible to increase the acceptability among the varying age cohorts who would likely benefit from the nutritional profile of this product.

4. Conclusion

Response surface methodology represents a useful tool to investigate the effects of four factors on the physicochemical characteristics of the restructured beef steaks. A restructured meat product with RP could be developed using the PiVac technology with a nutritional profile oriented to mitigate sarcopenia in older adults. The interactions observed between PPI, RP and LF appear to favour the gel-network formation, as much as transglutaminase indicating that it could be feasible to obtain a novel restructured beef steak with acceptable textural characteristics for older consumers using only clean label ingredients. This work underscores the utility of both *in vitro* digestion and microscopy as a way to visualise fibre separation, fibre breakdown and protein re-aggregation in a protein-enriched meat product after gastro-intestinal digestion. Finally, a consumer study on 120 older adults on the optimised restructured beef formulation showed that steaks containing plant-based ingredients or in combination with seasoning inclusions did undergo some modification in sensorial attributes and notably, these differences were not perceptible in the oldest cohort (80+ years). These results provide an understanding of the factors of importance in the development of protein-enriched restructured beef steaks for this cohort.

Acknowledgements

This research was funded by the FIRM programme administered under the Irish Department of Agriculture, Food and the Marine, Meat4Vitality: Enhancement of texture, flavour and nutritional value of meat products for older adults and the Walsh Fellowship Scheme (11/F/045). The authors also wish to acknowledge the contributions of Eugene Vesey, Karen Hussey, Sarah Lynch, Cristina Botineştean and Paula Reid. The authors acknowledge the technical and statistical assistance received from David Sanderson, Terence Hagan and Alan W. Gordon for the consumer study carried out at the Agri-Food and Biosciences Institute, Belfast.

References

Adebiyi, A. P., & Aluko, R. E. (2011). Functional properties of protein fractions obtained from commercial yellow field pea (Pisum sativum L.) seed protein isolate. *Food Chemistry, 128*(4), 902-908. doi: http://dx.doi.org/10.1016/j.foodchem.2011.03.116

Akcan, T., Estévez, M., & Serdaroğlu, M. (2017). Antioxidant protection of cooked meatballs during frozen storage by whey protein edible films with phytochemicals from Laurus nobilis L. and Salvia officinalis. *LWT - Food Science and Technology, 77*, 323-331. doi: http://dx.doi.org/10.1016/j.lwt.2016.11.051

Akwetey, W. Y., & Knipe, C. L. (2012). Sensory attributes and texture profile of beef burgers with gari. *Meat Science*, 92(4), 745-748. doi: https://doi.org/10.1016/j.meatsci.2012.06.032

Alakali, J. S., Irtwange, S. V., & Mzer, M. T. (2010). Quality evaluation of beef patties formulated with bambara groundnut (Vigna subterranean L.) seed flour. *Meat Science, 85*(2), 215-223. doi: http://dx.doi.org/10.1016/j.meatsci.2009.12.027

Aleson-Carbonell, L., Fernández-López, J., Pérez-Alvarez, J. A., & Kuri, V. (2005). Characteristics of beef burger as influenced by various types of lemon albedo. *Innovative Food Science & Emerging Technologies*, 6(2), 247-255. doi: https://doi.org/10.1016/j.ifset.2005.01.002

Baugreet, Hamill, R., Allen, P., & Kerry, J. P. (2017). Application of the novel PiVac technology to develop a fortified portion-controlled restructured beef steak *Journal of Food Processing and Preservation*.

Baugreet, Hamill, R. M., Kerry, J. P., & McCarthy, S. N. (2017). Mitigating Nutrition and Health Deficiencies in Older Adults: A Role for Food Innovation? *Journal of Food Science*, doi: 10.1111/1750-3841.13674

Baugreet, Kerry, J. P., Botineştean, C., Allen, P., & Hamill, R. M. (2016). Development of novel fortified beef patties with added functional protein ingredients for the elderly. *Meat Science*. doi: http://dx.doi.org/10.1016/j.meatsci.2016.07.004

Beelen, J., De Roos, N. M., & De Groot, L. C. P. G. M. (2016). Protein enrichment of familiar foods as an innovative strategy to increase protein intake in institutionalized elderly. *The journal of nutrition, health & aging*, 1-7. doi: 10.1007/s12603-016-0733-y

Bourne, M. C. (1978). Texture profile Analysis. *Food Technology, 21*, 62-66 72.

Bouvard, V., Loomis, D., Guyton, K. Z., Grosse, Y., Ghissassi, F. E., Benbrahim-Tallaa, L., Guha, N., Mattock, H. & Straif, K. 2015. Carcinogenicity Of Consumption Of Red And Processed Meat. The Lancet Oncology, 16, 1599-1600.

Cetiner, B., Acar, O., Kahraman, K., Sanal, T., & Koksel, H. (2017). An investigation on the effect of heat-moisture treatment on baking quality of wheat by using response surface methodology. *Journal of Cereal Science, 74*, 103-111. doi: http://dx.doi.org/10.1016/j.jcs.2017.01.002

Colmenero, F. J., Ayo, M. J., & Carballo, J. (2005). Physicochemical properties of low sodium frankfurter with added walnut: effect of transglutaminase combined with caseinate, KCl and dietary fibre as salt replacers. *Meat Sci, 69*(4), 781-788. doi: 10.1016/j.meatsci.2004.11.011

Deer, R. R., & Volpi, E. (2015). Protein intake and muscle function in older adults. *Current Opinion in Clinical Nutrition and Metabolic Care, 18*(3), 248-253. doi: 10.1097/mco.0000000000000162

Gao, X., Zhang, W., & Zhou, G. (2014). Effects of glutinous rice flour on the physiochemical and sensory qualities of ground pork patties. *LWT - Food Science and Technology, 58*(1), 135-141. doi: http://dx.doi.org/10.1016/j.lwt.2014.02.044

Gariballa, S., & Alessa, A. (2013). Sarcopenia: Prevalence and prognostic significance in hospitalized patients. *Clinical Nutrition, 32*(5), 772-776. doi: http://dx.doi.org/10.1016/j.clnu.2013.01.010

Griep, M. I., Mets, T. F., & Massart, D. L. (1996). Different effects of flavour amplification of nutrient dense foods on preference and consumption in young and elderly subjects. *Food Quality and Preference, 8*(2), 151-156.

Griep, M. I., Mets, T. F., & Massart, D. L. (2000). Effects of flavour amplification of Quorn (R) and yoghurt on food preference and consumption in relation to age, BMI and odour perception. *British Journal of Nutrition, 83*(2), 105-113.

Hayes, J. E., Stepanyan, V., Allen, P., O'Grady, M. N., & Kerry, J. P. (2011). Evaluation of the effects of selected plant-derived nutraceuticals on the quality and shelf-life stability of raw and cooked pork sausages. *LWT - Food Science and Technology, 44*(1), 164-172. doi: http://dx.doi.org/10.1016/j.lwt.2010.05.020

Holman, B. W. B., van de Ven, R. J., Mao, Y., Coombs, C. E. O., & Hopkins, D. L. (2017). Using instrumental (CIE and reflectance) measures to predict consumers' acceptance of beef colour. *Meat Science, 127*, 57-62. doi: http://dx.doi.org/10.1016/j.meatsci.2017.01.005

ISO 936:, I. S. O. (1998). Meat and meat products - Determination of total ash: International Standards Organization.

ISO. (2008). ISO 8589 Sensory analysis - General guidance for the design of test rooms. International Standards Organization.

Kappeler, R., Eichholzer, M., & Rohrmann, S. (2013). Meat consumption and diet quality and mortality in NHANES III. *Eur J Clin Nutr, 67*(6), 598-606. doi: 10.1038/ejcn.2013.59

Lepper-Blilie, A. N., Berg, E. P., Germolus, A. J., Buchanan, D. S., & Berg, P. T. (2014). Consumer evaluation of palatability characteristics of a beef value-added cut compared to common retail cuts. *Meat Science, 96*(1), 419-422. doi: http://dx.doi.org/10.1016/j.meatsci.2013.08.002

Mathey, M.-F. A. M., Siebelink, E., de Graaf, C., & Van Staveren, W. A. (2001). Flavor Enhancement of Food Improves Dietary Intake and Nutritional Status of Elderly Nursing Home Residents. *The Journals of Gerontology: Series A, 56*(4), M200-M205. doi: 10.1093/gerona/56.4.M200

Meyers, R. H., & Montgomery, D. C. (2002). *Response Surface Methodology* (2nd Ed. ed.). New York: John Wiley & Sons, Inc,.

Mintel. (2013). % share of clean label new product launches from new product launches in packaged food per country in 2013.

Nehete, J. Y., Bhambar, R. S., Narkhede, M. R., & Gawali, S. R. (2013). Natural proteins: Sources, isolation, characterization and applications. *Pharmacognosy Reviews, 7*(14), 107-116. doi: 10.4103/0973-7847.120508

Orla, K., Barbara, S. K., Peter, M., & David, T. (2004). The influence of context upon consumer sensory evaluation of chicken-meat quality. *British Food Journal*, 106(3), 158-165. doi: 10.1108/00070700410528754

Petracci, M., Bianchi, M., Mudalal, S., & Cavani, C. (2013). Functional ingredients for poultry meat products. *Trends in Food Science & Technology, 33*(1), 27-39. doi: http://dx.doi.org/10.1016/j.tifs.2013.06.004

Rohrmann, S., Overvad, K., Bueno-de-Mesquita, H. B., Jakobsen, M. U., Egeberg, R., Tjønneland, A., Linseisen, J. (2013). Meat consumption and mortality - results from the European Prospective Investigation into Cancer and Nutrition. *BMC Medicine, 11*(1), 63. doi: 10.1186/1741-7015-11-63

Schoufour, J. D., Voortman, T., Franco, O. H., & Kiefte-De Jong, J. C. (2017). Chapter 11 - Dietary Patterns and Healthy Aging A2 - Raats, Monique M. In L. C. P. G. M. d. Groot & D. v. Asselt (Eds.), *Food for the Aging Population (Second Edition)* (pp. 223-254): Woodhead Publishing.

Singh, J., Kaur, L., & Singh, H. (2013). Chapter Four - Food Microstructure and Starch Digestion. In J. Henry (Ed.), *Advances in Food and Nutrition Research* (Vol. 70, pp. 137-179): Academic Press.

Siu, G. M., & Draper, H. H. (1978). A survey of the malonaldehyde content of retail meats and fish. *Journal of Food Science, 43*(4), 1147-1149. doi: 10.1111/j.1365-2621.1978.tb15256.x

Stelten, S., Dekker, I. M., Ronday, E. M., Thijs, A., Boelsma, E., Peppelenbos, H. W., & de van der Schueren, M. A. E. (2015). Protein-enriched 'regular products' and their effect on protein intake in acute hospitalized older adults; a randomized controlled trial. *Clinical Nutrition*(0). doi: http://dx.doi.org/10.1016/j.clnu.2014.08.007

Sun, X. D. (2009). Utilization of restructuring technology in the production of meat products: a review. *Cyta-Journal of Food, 7*(2), 153-162. doi: 10.1080/19476330903010193

Taylor, J. M., & Hopkins, D. L. 2011. Patents for Stretching and Shaping Meats. Recent Patents on Food, Nutrition & Agriculture, 3, 91-101.

Tobin, B. D., O'Sullivan, M. G., Hamill, R. M., & Kerry, J. P. (2012). Effect of varying salt and fat levels on the sensory quality of beef patties. *Meat Science, 91*(4), 460-465. doi: http://dx.doi.org/10.1016/j.meatsci.2012.02.032

Ustunol, Z. (2014). *Applied Food Protein Chemistry*: Wiley.

Valenzuela Melendres, M., Camou, J. P., Torrentera Olivera, N. G., Álvarez Almora, E., González Mendoza, D., Avendaño Reyes, L., & González Ríos, H. (2014). Response surface methodology for predicting quality characteristics of beef patties added with flaxseed and tomato paste. *Meat Science, 97*(1), 54-61. doi: http://dx.doi.org/10.1016/j.meatsci.2014.01.007

Yusop, S. M., O'Sullivan, M. G., Kerry, J. F., & Kerry, J. P. (2012). Influence of processing method and holding time on the physical and sensory qualities of cooked marinated chicken breast fillets. *LWT - Food Science and Technology, 46*(1), 363-370. doi: https://doi.org/10.1016/j.lwt.2011.08.007

Table 1 Experimental range and values of the independent process variables in the central composite design

Independent variables	Symbol	Levels				
		-α	**-1**	**0**	**+1**	**+α**
Transglutaminase (U/g)	X_1	0.25	1	1.75	2.5	3.25
Pea protein Isolate (g)	X_2	4.25	5.5	6.75	8	9.25
Rice protein (g)	X_3	6.25	7.5	8.75	10	11.25
Lentil flour (g)	X_4	0.25	1.5	2.75	4	5.25

Design factors are transglutaminase (X_1: TG), pea protein isolate (X_2: PPI), rice protein (X_3: RP) and lentil flour (X_4: LF). Values are expressed as g/100g of meat.

Table 2 Sensory descriptors for the untrained panellist evaluation of cooked restructured steaks

Attribute	Description
Colour	0 = pale, 10 = dark
Aroma	0 = extremely weak, 10 = extremely strong

Toughness	0 = extremely tough, 10 = extremely tender
Chewiness	0 = not chewy, 10 = extremely chewy
Juiciness	0 = extremely dry, 10=extremely juicy
Greasiness	0 = not greasy, 10 =extremely greasy
Flavour acceptability	0 = extremely like, 10 = extremely dislike
Overall acceptability	0 = extremely unacceptable, 10 = extremely acceptable

Table 3 ANOVA results for the experimental variables of each response variable and corresponding coefficients for the predictive models

Source	**DF**	**Compositional parameters**									
		Raw				**Cooked**					
		Y_1		**Y_2**		**Y_3**		**Y_4**		**Y_5**	
		Coef.	**p-value**	**Coef.**	**p-value**	**Coef.**	**p-value**	**Coef.**	**p-value**	**Coef.**	**p-value**
Intercept	14	66.6	0.012	27.0	0.014	63.8	0.034	28.2	0.096	4.31	0.045
X_1	1	-0.872	0.058	-	-	-	-	-	-	-	-
X_2	1	0.295	0.507	-	-	-	-	-	-	-0.254	0.361
X_3	1	-1.32	0.006	0.843	0.014	-1.06	0.034	0.646	0.096	0.118	0.668
X_4	1	-0.703	0.122	-	-	-	-	-	-	-	-
X_2X_3	1	-	-	-	-	-	-	-	-	-0.946	0.008
Residual	15										
Lack of	10		0.569		0.486		0.301		0.116		0.305

fit						
Pure error	5					
total	29					
R^2		0.390	0.196	0.151	0.096	0.262
Adj-R^2		0.293	0.167	0.120	0.064	0.177

$\mathbf{Y_1}$: moisture (raw), $\mathbf{Y_2}$: protein (raw), $\mathbf{Y_3}$: moisture (cooked), $\mathbf{Y_4}$: protein (cooked), $\mathbf{Y_5}$: fat (cooked), $\mathbf{X_1}$: Transglutaminase, $\mathbf{X_2}$: Pea protein isolate, $\mathbf{X_3}$: Rice protein, $\mathbf{X_4}$: Lentil flour

Table 4 ANOVA results for the experimental variables of each response variable and corresponding coefficients for the predictive models

Source	**DF**	**Cooking loss**		**Lipid oxidation**				**Textural parameters**						
		Y_6		**Y_7**		**Y_8**		**Y_9**		**Y_{10}**		**Y_{11}**		**Y_{12}**
		Coef.	**p-value**	**Coef.**	**P-value**	**Coef.**	**P-value**	**Coef.**	**p-value**	**Coef.**	**p-value**	**Coef.**	**p-value**	**Co**
Intercept	14	15.5	0.002	0.424	0.006	0.578	0.016	966	0.048	0.794	0.002	2164	0.017	21
X_1	1	0.198	0.790	-	-	-	-	-	-	-0.003	0.427	395	0.027	-
X_2	1	-0.385	0.606	-0.009	0.764	0.051	0.084	-10.1	0.738	-	-	-	-	-
X_3	1	-2.84	0.001	0.014	0.659	-0.059	0.050	-	-	-0.004	0.296	-	-	-
X_4	1	-2.06	0.010	-	-	-0.012	0.674	-65.3	0.038	-0.008	0.074	-338	0.055	-30
X_1X_2	1	-	-	-	-	-	-	-	-	-	-	-	-	-
X_1X_3	1	-	-	-	-	-	-	-	-	0.009	0.072	-	-	-
X_1X_4	1	-	-	-	-	-	-	-	-	-	-	-	-	-
X_2X_3	1	-	-	0.105	0.009	-	-	-	-	-	-	-	-	-
X_2X_4	1	-	-	-	-	-	-	-74.9	0.051	-	-	-	-	-
X_3X_4	1	-	-	-	-	-	-	-	-	0.019	0.001	-	-	-

X_2^2	1	-	-	0.054	0.063	-	-	-	-	-	-	-	-	-
X_3^2	1	-	-	0.091	0.003	-	-	-	-	-0.010	0.011	-	-	-
X_4^2	1	-	-	-	-	0.071	0.012	-	-	-0.007	0.056	-	-	-
Residual	15													
Lack of fit	10		0.570		0.671		0.666		0.135		0.003		0.414	
Pure error	5													
total	29													
R^2			0.481		0.473		0.376		0.259		0.614		0.261	
Adj-R^2			0.398		0.363		0.277		0.173		0.492		0.206	

Y_6: Cooking loss, **Y_7**: lipid oxidation day 0, **Y_8**: lipid oxidation day 30, **Y_9**: hardness, **Y_{10}**: cohesiveness, **Y_{11}**: chewiness, **Y_{12}**: gumminess, **Y_{13}**: springiness, **Y_{14}**: bind strength, **X_1**: Transglutaminase, **X_2**: Pea protein isolate, **X_3**: Rice protein, **X_4**: Lentil flour

Table 5 ANOVA results for the experimental variables of each response variable and corresponding coefficients for the predictive model

Source	**DF**	**Restructured steaks colour parameters**					
		Raw				**Cooked**	
		Y_{15}		**Y_{16}**		**Y_{17}**	
		Coef.	**p-value**	**Coef.**	**p-value**	**Coef.**	**p-value**
Intercept	14	50.9	0.001	9.51	0.009	53.4	0.006
X_1	1	0.405	0.159	0.027	0.688	0.022	0.937
X_2	1	-0.433	0.134	-0.030	0.661	0.134	0.636
X_3	1	0.425	0.140	-0.012	0.865	0.943	0.002
X_4	1	0.922	0.003	-0.003	0.965	0.730	0.015
X_1X_2	1	-	-	-0.012	0.149	-	-
X_1X_3	1	-	-	-0.054	0.516	-	-
X_1X_4	1	-	-	0.254	0.006	-	-
X_2X_3	1	-	-	-0.280	0.003	-	-
X_2X_4	1	-	-	0.200	0.024	-	-
X_3X_4	1	-	-	-0.175	0.044	-	-
X_2^2	1	-	-	-	-	-	-
X_3^2	1	-	-	-	-	-	-
X_4^2	1	-	-	-	-	-	-
Residual	15						
Lack of fit	10		0.269		0.975		0.667
Pure error	5						
total	29						
R^2			0.415		0.650		0.000
Adj-R^2			0.322		0.467		0.000

Y_{15}: lightness (L*), **Y_{16}**: redness (a*), **Y_{17}**: lightness (L*)

Table 6 Predicted, experimental values, accuracy factor (AF), bias factor (BF) and average mean deviation (∑%) values for optimised formulation 1 and 2

Variables	Optimisation criteria	Formulation 1	Experimental Formulation 2	Accuracy Factor	Bias Factor	∑ (%)	Formulation 2	Experimental Formulation 2	Accuracy Factor	Bias Factor	∑ (%)
Independent											
X_1: TG	Target: 2	2	2				2	2			
X_2: PPI	Range: 6-8	8	8				8	8			
X_3: RP	Maximise	9.35	9.35				9.39	9.39			
X_4: LF	Range: 1.5-4	4.00	4.00				4.00	4.00			
Dependent Raw attribute											
Protein	Maximise	27.4	26.2	1.03	0.97	2.64	27.5	25.9	1.06	0.95	5.50
L*	Minimise	51.7	51.7	1.00	1.00	0.15	51.8	52.4	1.01	0.99	1.10
a*	Maximise	9.55	11.8	1.24	0.81	19.11	9.49	13.0	1.37	0.73	27.2
Cooked attribute											
Moisture	Maximise	63.9	64.3	1.01	0.99	1.39	63.3	65.9	1.01	0.99	1.47
Protein	Maximise	28.5	29.7	1.04	0.96	4.09	28.6	28.6	1.00	1.00	0.09
Hardness	Minimise	815	811	0.99	1.01	0.50	815	812	1.00	1.00	0.43
Cohesiveness	Range: 0.73-0.84	0.78	0.75	0.96	1.04	3.95	0.79	0.78	1.00	1.00	0.01
Chewiness	Minimise	1957	1986	1.02	0.99	1.49	1957	1949	1.00	1.00	0.39
Gumminess	Minimise	183	166	0.91	1.10	10.0	184	163	0.89	1.13	12.8

Springiness	Range: 5.59-12.17	10.0	10.2	1.02	0.98	1.74	10.01	10.4	1.04	0.97	3.49
Bind Strength	Target: 6369	7095	7093	1.00	1.00	0.03	7095	7067	1.00	1.00	0.39
Cook loss	Minimise	12.1	11.6	0.96	1.04	3.90	11.7	11.9	1.02	0.98	1.83
L*	Minimise	54.7	57.9	1.06	0.94	5.64	54.8	58.6	1.07	0.94	6.43
a*	Range: 2.34-3.93	3.35	5.12	1.53	0.65	34.6	3.35	4.54	1.35	0.74	26.14
Desirability		**0.57**					**0.57**				

Table 7 Sensory attributes of optimised formulations (with and without seasonings) compared to controls

Parameters	Formulations	Seasonings Without	Formulations	Seasonings With	Source of variation	*P* value
Colour	**C**	6.18^{abX}	**CS**	7.29^{bY}	**Formulation**	0.015
	F1	5.09^{aX}	**F1S**	5.73^{abY}	**Seasoning**	0.041
	F2	5.59^{abX}	**F2S**	6.16^{abY}	**Form x Seas**	0.816
Aroma	**C**	3.03^{a}	**CS**	3.86^{ab}	**Formulation**	0.001
	F1	6.11^{b}	**F1S**	6.14^{b}	**Seasoning**	0.485
	F2	6.14^{b}	**F2S**	6.36^{b}	**Form x Seas**	0.800
Toughness	**C**	5.84^{X}	**CS**	7.09^{Y}	**Formulation**	0.478
	F1	5.40^{X}	**F1S**	6.51^{Y}	**Seasoning**	0.002
	F2	4.97^{X}	**F2S**	6.78^{Y}	**Form x Seas**	0.785
Chewiness	**C**	4.47^{X}	**CS**	4.53^{Y}	**Formulation**	0.070
	F1	4.54^{X}	**F1S**	3.27^{Y}	**Seasoning**	0.048
	F2	3.95^{X}	**F2S**	2.64^{Y}	**Form x Seas**	0.321
Juiciness	**C**	4.46^{b}	**CS**	4.35^{b}	**Formulation**	0.001
	F1	2.36^{a}	**F1S**	2.38^{a}	**Seasoning**	0.827
	F2	2.02^{a}	**F2S**	1.85^{a}	**Form x Seas**	0.981
Greasiness	**C**	1.81^{ab}	**CS**	3.02^{ab}	**Formulation**	0.054
	F1	1.22^{a}	**F1S**	2.17^{ab}	**Seasoning**	0.058
	F2	1.52^{ab}	**F2S**	1.33^{ab}	**Form x Seas**	0.212
Flavour Acceptability	**C**	2.42^{a}	**CS**	3.98^{ab}	**Formulation**	0.001
	F1	6.61^{c}	**F1S**	5.41^{bc}	**Seasoning**	0.353
	F2	6.33^{bc}	**F2S**	4.57^{abc}	**Form x Seas**	0.018

Overall acceptability	**C**	6.84^{b}	**CS**	5.54^{ab}	**Formulation**	0.001
	F1	3.57^{a}	**F1S**	3.90^{a}	**Seasoning**	0.528
	F2	3.26^{a}	**F2S**	3.32^{a}	**Form x Seas**	0.336

^{abc} Means in the same column for each parameter (different formulations) that do not share a common superscript are significantly different according to Fisher's Protected Least Significant Difference (FPLSD) ($P<0.05$)

^{XY} Means in the same row (Seasoning) that do not share a common superscript are significantly different ($P<0.05$)

Form: Formulation

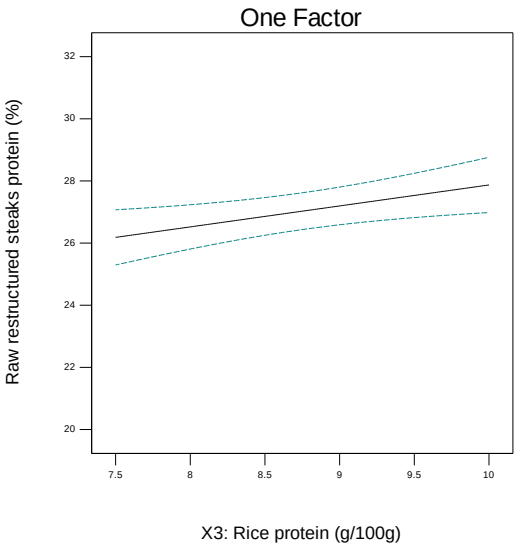
Seas: Seasoning

a)

Design-Expert® Software
Factor Coding: Actual
ProteinR
95% CI Bands

X1 = C: RP

Actual Factors
A: TG = 1.75
B: PPI = 5.5
D: LF = 2.75



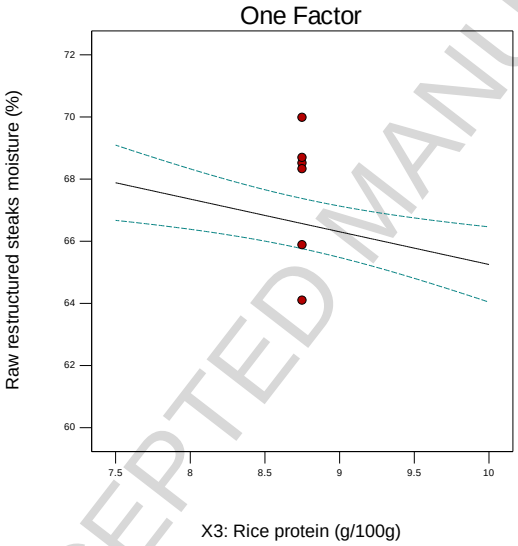
b)

c)

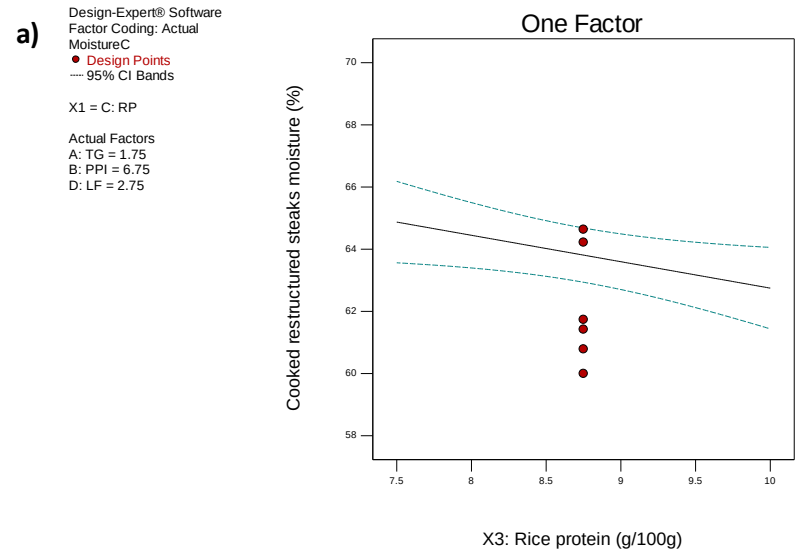
Design-Expert® Software
Factor Coding: Actual
MoistureR
Design Points
95% CI Bands

X1 = C: RP

Actual Factors
A: TG = 1.75
B: PPI = 6.75
D: LF = 2.75



d)



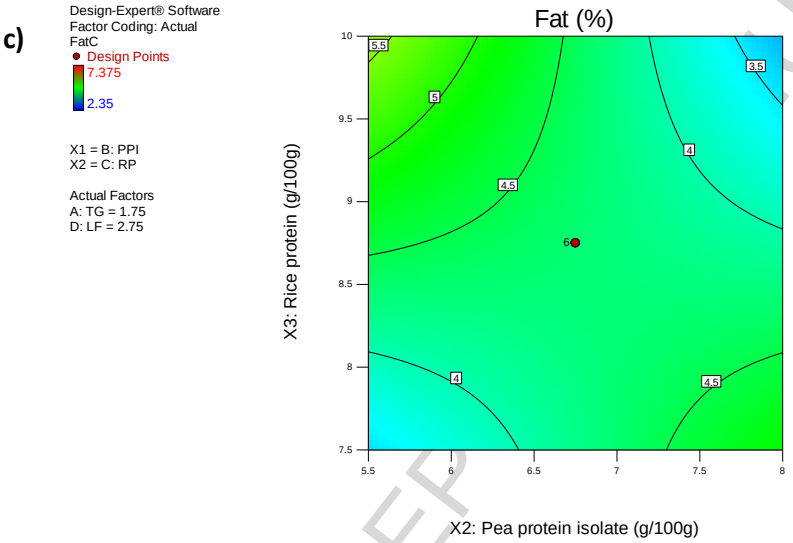
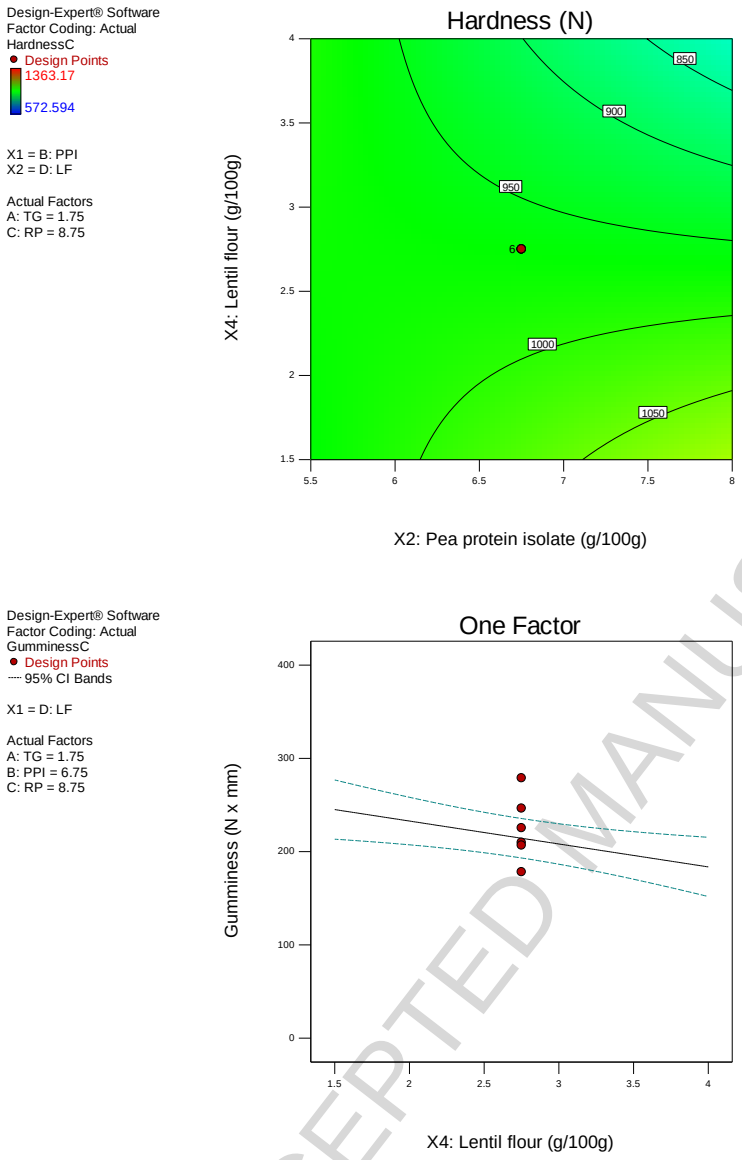


Fig. 1 Representation of the surface models for raw protein (a), raw moisture (b), cooked moisture (c) and cooked fat (d) on restructured steaks

a)



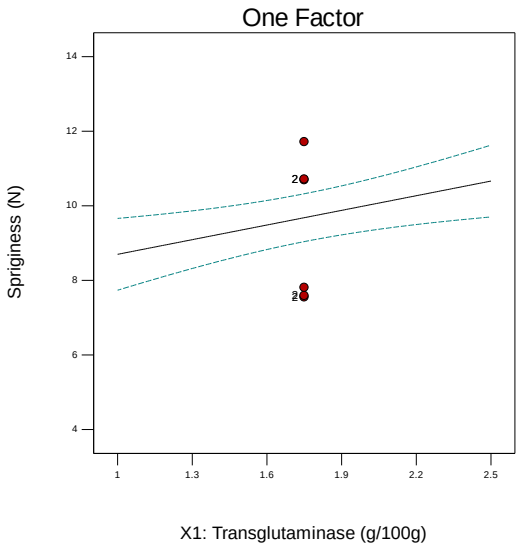
b)

c)

Design-Expert® Software
Factor Coding: Actual
SpriginessC
● Design Points
····· 95% CI Bands

X1 = A: TG

Actual Factors
B: PPI = 6.75
C: RP = 8.75
D: LF = 2.75



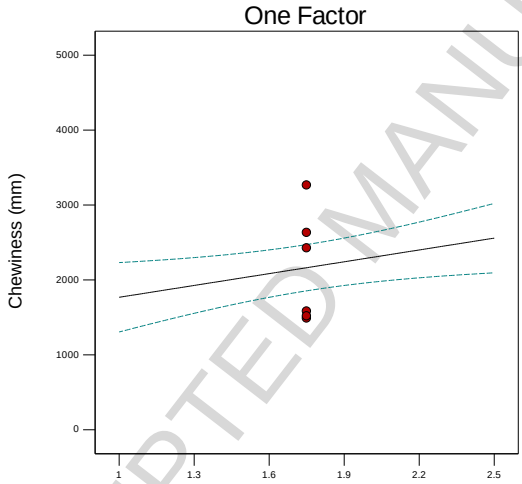
X1: Transglutaminase (g/100g)

d)

Design-Expert® Software
Factor Coding: Actual
ChewinessC
● Design Points
····· 95% CI Bands

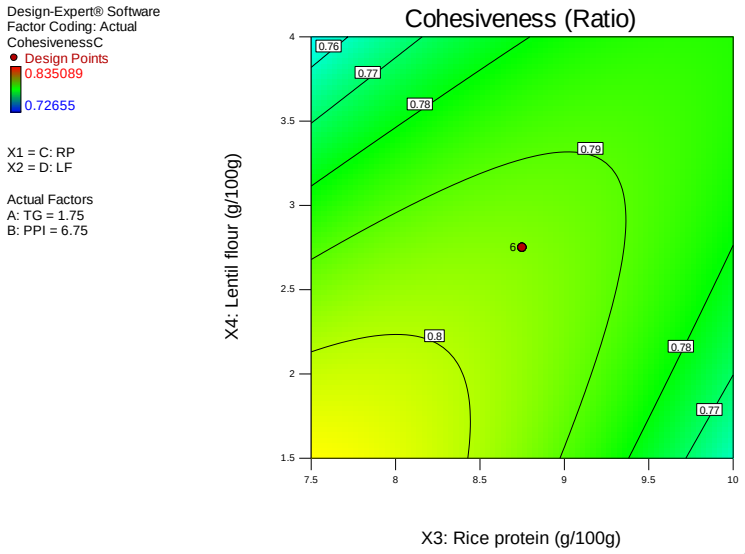
X1 = A: TG

Actual Factors
B: PPI = 6.75
C: RP = 8.75
D: LF = 2.75



X1: Transglutaminase (g/100g)

e)



f)

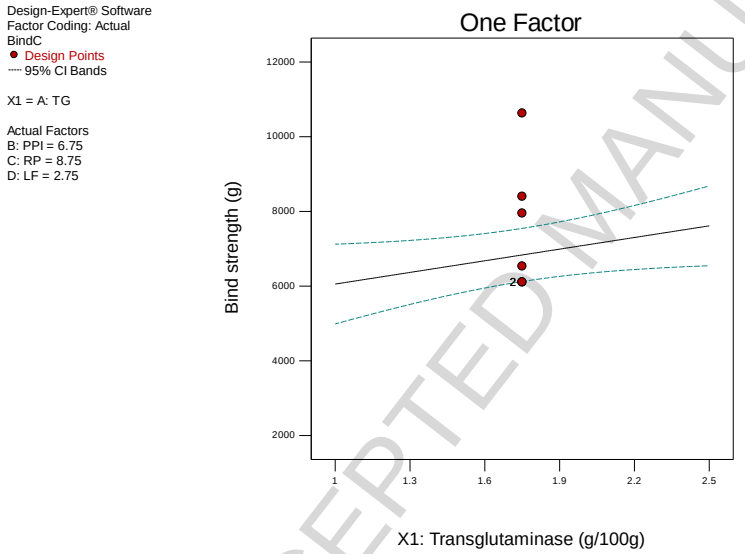


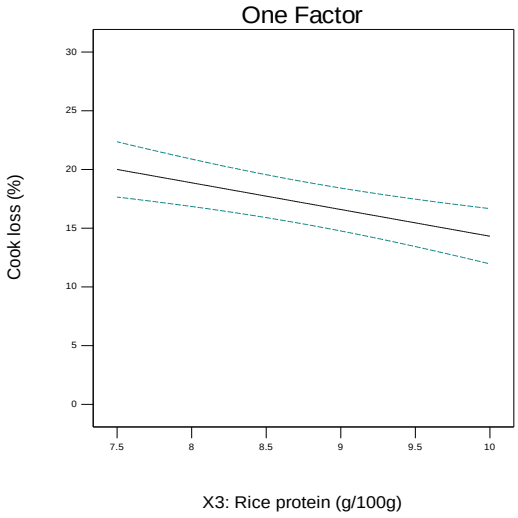
Fig. 2 Representation of the surface models for hardness (a), gumminess (b), springiness (c), chewiness (d), cohesiveness (e) and bind strength (f)

a)

Design-Expert® Software
Factor Coding: Actual
Cook loss
----- 95% CI Bands

X1 = C: RP

Actual Factors
A: TG = 1.75
B: PPI = 6.75
D: LF = 1.77027

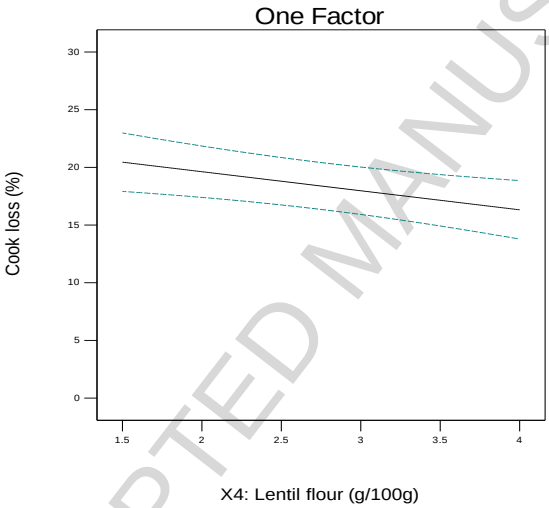


b)

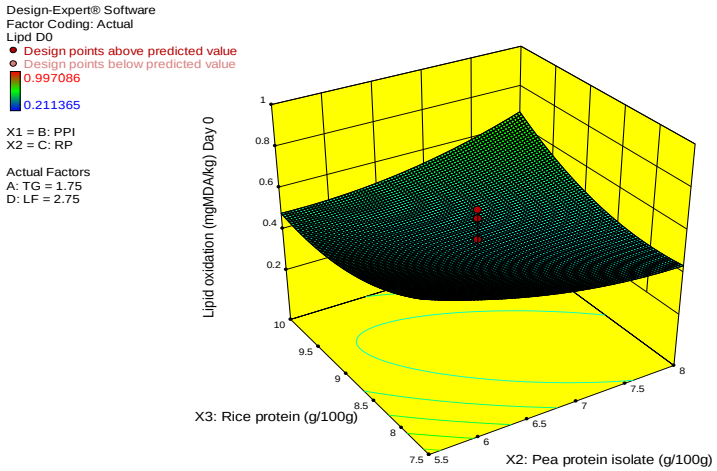
Design-Expert® Software
Factor Coding: Actual
Cook loss
----- 95% CI Bands

X1 = D: LF

Actual Factors
A: TG = 1.75
B: PPI = 6.75
C: RP = 7.5



c)



d)

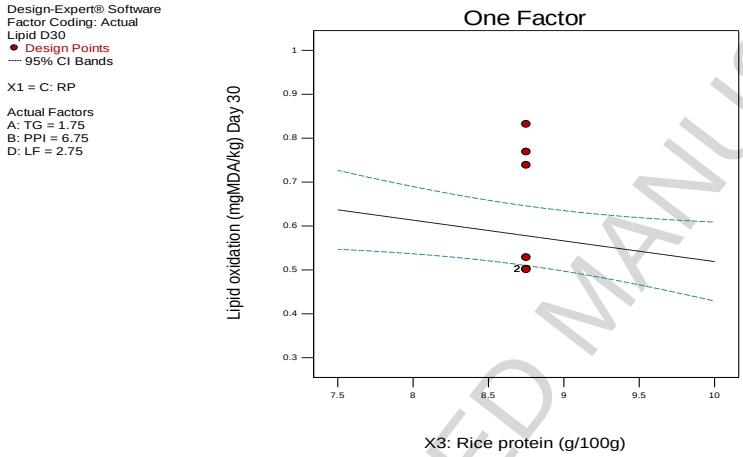


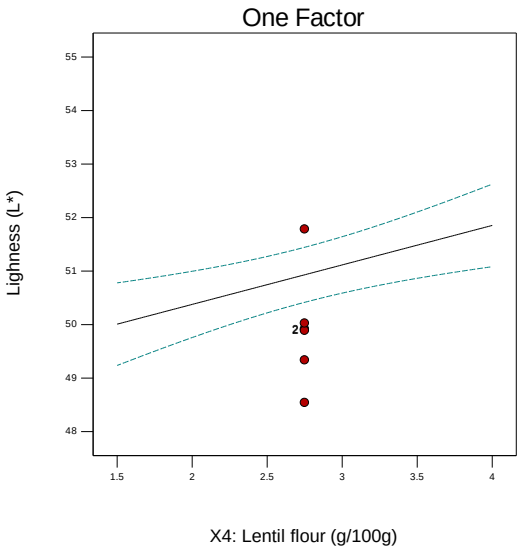
Fig. 3 Representation of the surface models for cooking loss (a, b) and lipid oxidation day 0 (c) – as a function of rice protein and pea protein isolate and day 30 (d)

a)

Design-Expert® Software
Factor Coding: Actual
R_L*
Design Points
95% CI Bands

X1 = D: LF

Actual Factors
A: TG = 1.75
B: PPI = 6.75
C: RP = 8.75

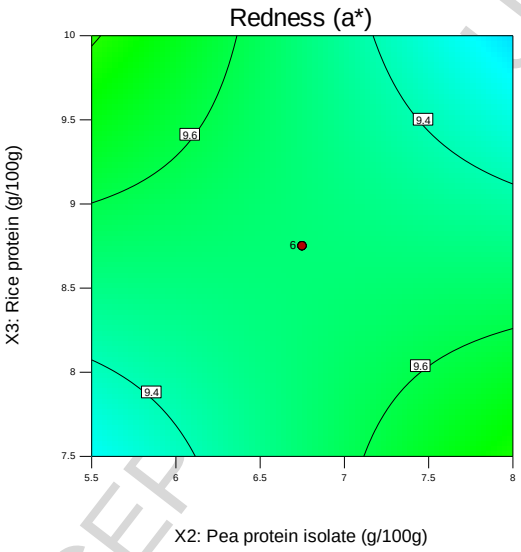


b)

Design-Expert® Software
Factor Coding: Actual
R_a*
Design Points
10.6958
8.78

X1 = B: PPI
X2 = C: RP

Actual Factors
A: TG = 1.75
D: LF = 2.75

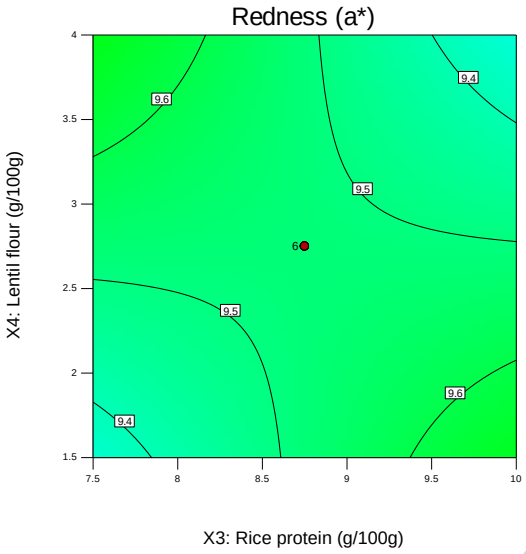


c)

Design-Expert® Software
Factor Coding: Actual
R_a*
Design Points
10.6958
8.78

X1 = C: RP
X2 = D: LF

Actual Factors
A: TG = 1.75
B: PPI = 6.75

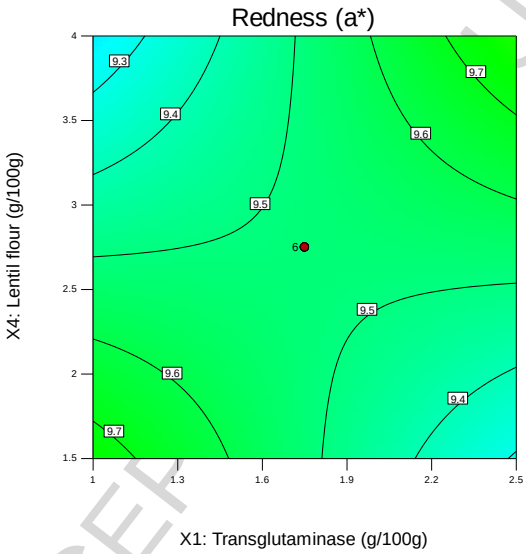


d)

Design-Expert® Software
Factor Coding: Actual
R_a*
Design Points
10.6958
8.78

X1 = A: TG
X2 = D: LF

Actual Factors
B: PPI = 6.75
C: RP = 8.75

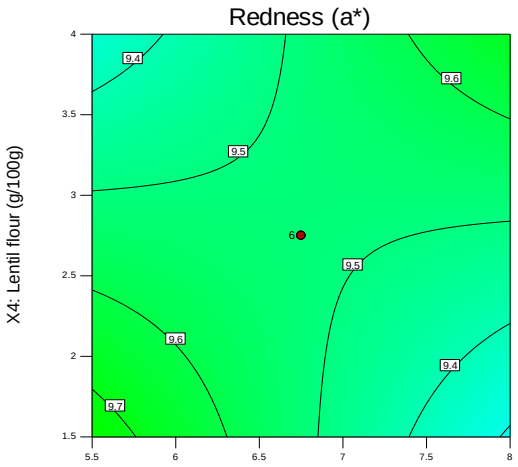


e)

Design-Expert® Software
Factor Coding: Actual
R_a*
Design Points
10.6958
8.78

X1 = B: PPI
X2 = D: LF

Actual Factors
A: TG = 1.75
C: RP = 8.75

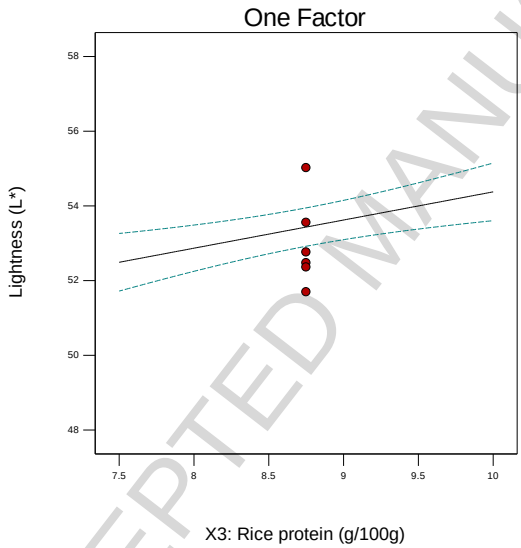


f)

Design-Expert® Software
Factor Coding: Actual
C_L*
Design Points
95% CI Bands

X1 = C: RP

Actual Factors
A: TG = 1.75
B: PPI = 6.75
D: LF = 2.75



g)

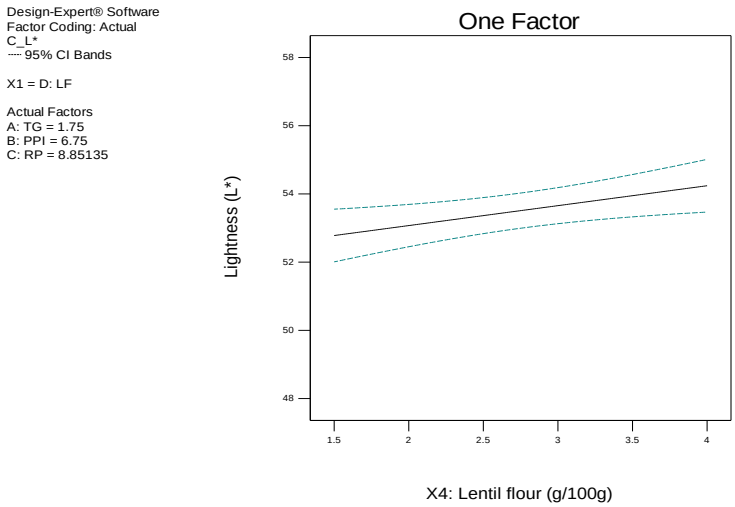


Fig. 4 Representation of the surface models for L* (a) and a* (b-e) for raw restructured steaks and L* (f,g) for cooked restructured steaks

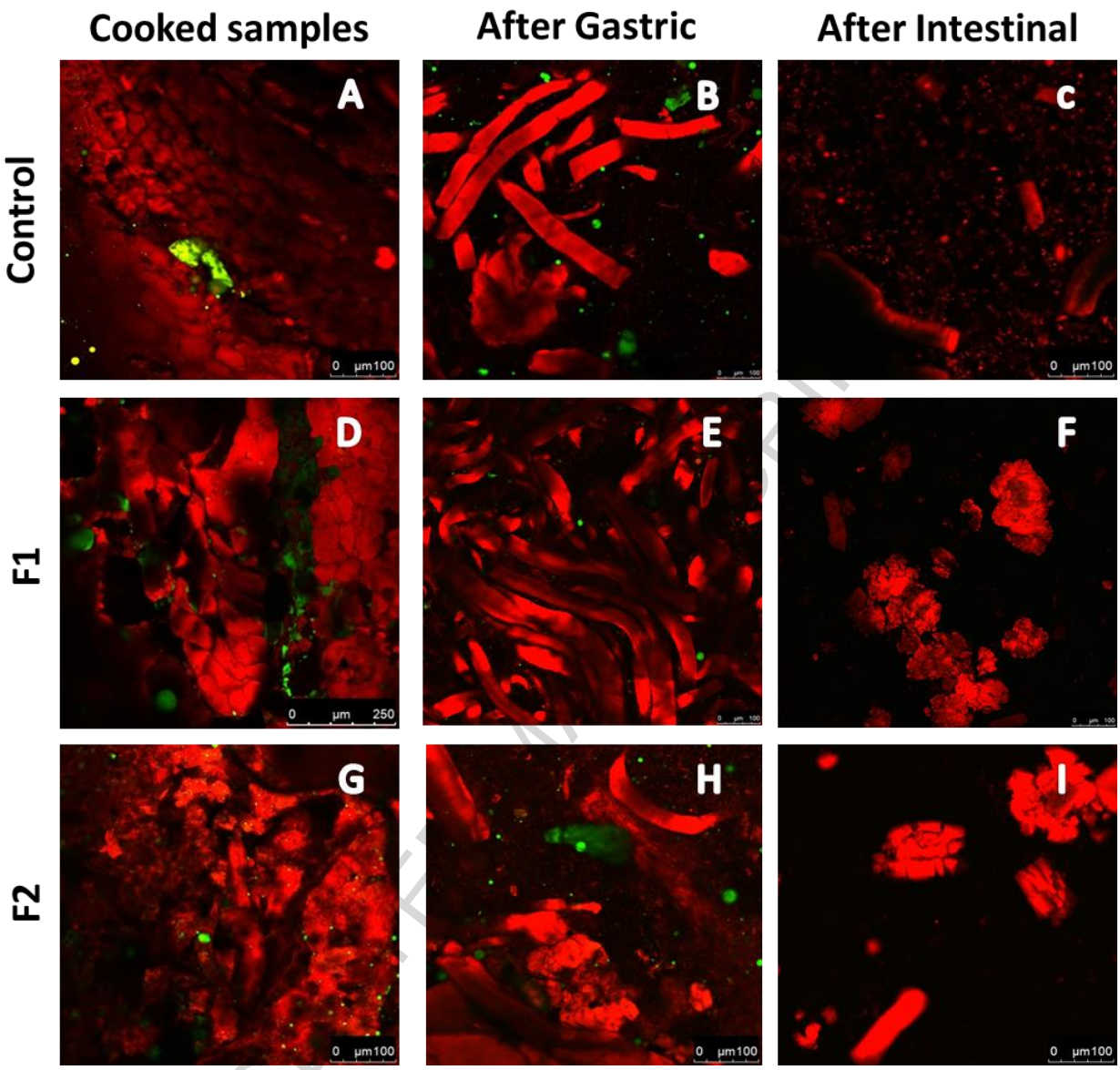
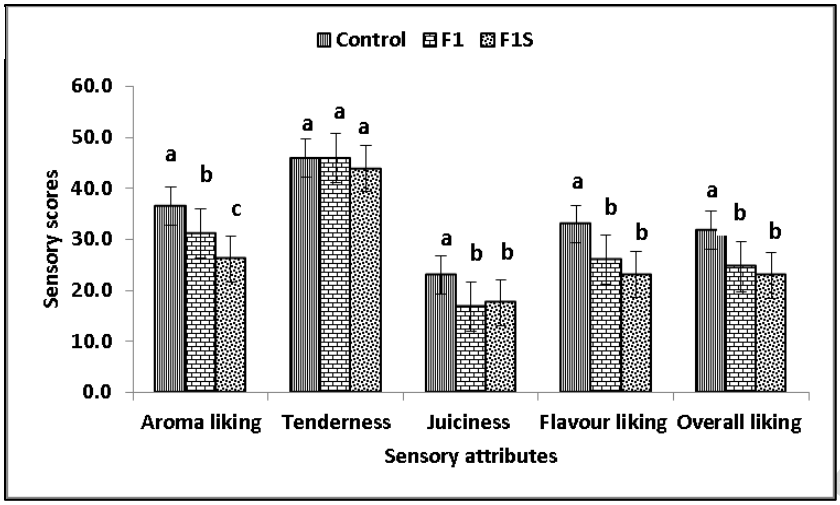
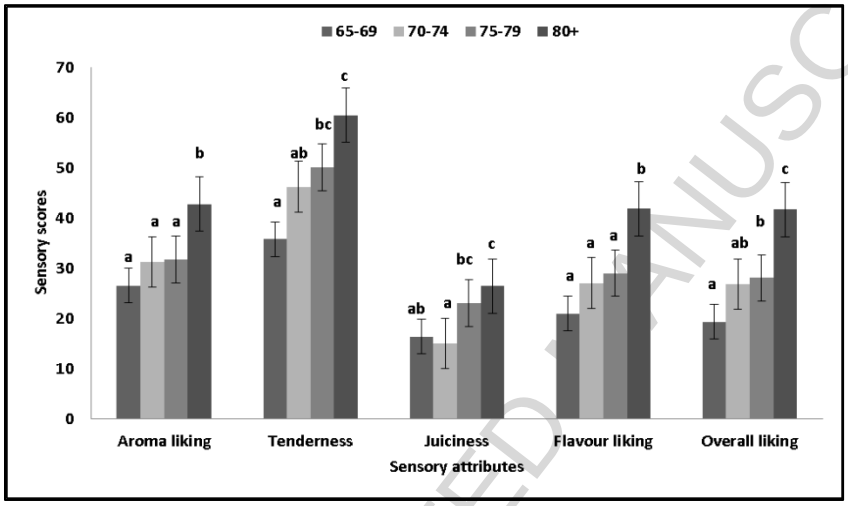


Fig. 5 Confocal scanning laser microscopy images of protein enriched restructured beef steaks

a)





c)

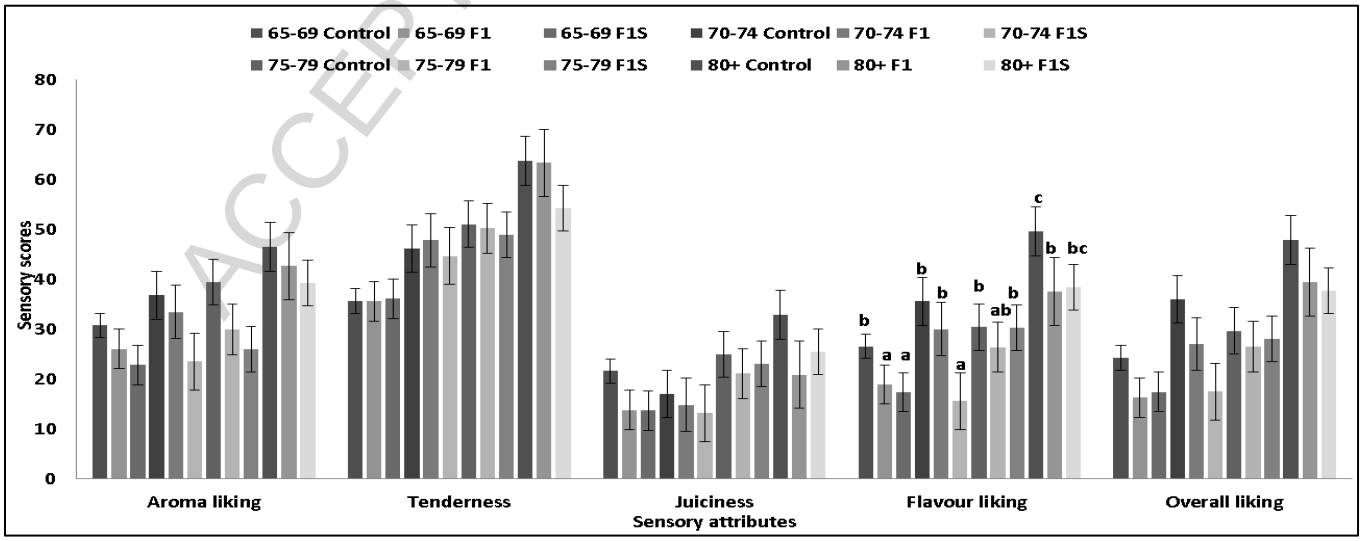


Fig. 6 Sensory attributes of restructured beef steaks; optimised formulation 1 (**F1**), optimised formulation with added seasonings (**F1S**) **(6a)**; Sensory attributes scores over the varying age cohorts

(65-69, 70-74, 75-79 and 80+) **(6b)**; Mean sensory scores of sensorial attributes in control, F1 and F1S by the varying age cohorts **(6c)**.[a-c] Mean values with different letters are significantly different ($P < 0.05$)

- A novel restructured beef steak was developed using pea protein isolate, rice protein and lentil flour.
- A protein content of 28g/100g was achieved, which could be beneficial for the older consumers.
- Inclusion of lentil flour had a positive effect on the textural parameters of restructured beef steaks
- A sensory consumer study of 120 older adults was carried to determine acceptability of the developed products.